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**GRANT#:** N00014-00-1-0663

**PRINCIPAL INVESTIGATOR:** Dr. Scott N. Peterson

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**GRANT TITLE:** Anthrax Gene Expression: Analysis of the Early Stages of Infection

**AWARD PERIOD:** 7/01/00-9/30/03

**ABSTRACT:**

Control of anthrax toxin and capsule synthesis, the two major virulence factors of *Bacillus anthracis*, has been associated with two regulatory genes, *atxA* and *acpA*, located on virulence plasmids pXO1 and pXO2, respectively. We used DNA microarrays to determine which genes in the *B. anthracis* genome are controlled by *atxA* and/or *acpA*. The regulation of numerous genes present on the virulence plasmids was documented and both positive and negative effects were observed. Certain *atxA*-regulated genes were affected synergistically in an *atxA*, *acpA* double mutants. *AcpA* appears to be a minor regulator with fewer targets than *atxA*. The newly discovered *atxA*-regulated targets include genes predicted to encode secreted proteins and proteins with roles in transcriptional regulation and signaling.

**KEY WORDS:**

DNA microarrays, gene expression, sporulation, virulence gene regulators

**OBJECTIVE:**

We wished to determine which genes in the *B. anthracis* genome are transcriptionally regulated by either *atxA* and/or *acpA* beyond those previously identified, namely the toxins, *pagA*, *lef*, and *cya* and various genes encoding for capsular biosynthesis functions. Taken together, this repertoire of genes comprise the most important virulence determinants for *B. anthracis* pathogenicity.

We also wished to explore the functional similarities (if any) that exist between the two transcription factors under investigation.

**APPROACH:**

The approach undertaken at TIGR was to construct a DNA microarray containing PCR amplified fragments of the open reading frames (ORFs) present within the partially completed *B. anthracis* genome sequence. In total PCR products representing 3,428 ORFs (3,290 chromosomal and 138 plasmid) were successfully amplified and spotted onto microarrays used in subsequent studies. This DNA microarray was to serve as the substrate for performing whole genome transcriptional profiling of *B. anthracis* in relevant environmental contexts that mimic the host environment

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during early stages of infection. However, given the limitation of experimentally relevant RNAs provided from Dr. Hanna's laboratory, we were permitted, in collaboration with Theresa Koehler's laboratory, to validate the newly created DNA microarray using alternative RNAs bearing substantial interest to the *B. anthracis* research community. We used transcriptional profiling to determine whether *atxA* and/or *acpA* control genes other than those already described, and whether functional similarities exist between these two regulators. Transcription was assessed in a pXO1<sup>+</sup> pXO2<sup>+</sup> parent strain and in isogenic mutants deleted for one or both regulatory genes.

## ACCOMPLISHMENTS:

We were successful in identifying the set of genes in the *B. anthracis* genome that are regulated by the transcriptional regulators, *atxA* and/or *acpA*. *AtxA*, regulates the majority and certainly the most important virulence determinants required for *B. anthracis* virulence and pathogenicity. The importance of the known targets of *atxA* suggest that identifying the complete set of genes regulated by this transcription factor will point interested investigators towards a greater set of genes with similarly important function in virulence and host infection. The *atxA* and *acpA* regulons have been defined through this effort.

## CONCLUSIONS:

Our analysis of the transcriptional profiles of a genetically complete (pXO1<sup>+</sup> pXO2<sup>+</sup>) toxigenic capsulated *B. anthracis* strain and isogenic mutants deleted for the regulatory genes indicates that these regulators affect expression of numerous *B. anthracis* genes on the plasmids and on the chromosome. The newly discovered *atxA*-regulated genes may have direct or indirect roles in *B. anthracis* pathogenesis. Some of the highly-regulated target genes on the plasmids are predicted to encode proteins associated with the *B. anthracis* cell membrane or cell wall biosynthesis. Our data reveal two significant findings regarding the relationship between these regulators. First, *atxA* positively regulates *acpA* expression, while *acpA* does not affect *atxA* expression. Second, the two regulators have a synergistic effect on expression of certain target genes. Our data expand the list of *atxA* and *acpA*-regulated genes in *B. anthracis*. Considering that all previously known targets of *atxA* are under positive regulation, it is somewhat surprising that *atxA* also negatively regulates a number of genes. *AtxA* may function as both a repressor and an activator, as has been reported for some transcriptional regulators. Moreover, some or all of the target genes may be controlled by an unidentified downstream regulator. Certain *atxA*-regulated genes identified in our experiments are predicted to encode proteins with similarity to known regulators. For example, the *atxA*-regulated gene pXO2-53, is predicted to encode a protein with sequence similarity to *AtxA* and *AcpA*. Another candidate for a downstream regulator is *pagR*. There is increasing evidence suggesting that the distinct phenotypes of the species with regard to pathogenesis may be due in some part to differential gene expression related to the presence or absence of functional regulatory proteins. The chromosomal regulator *plcR* of *B. thuringiensis* and *B. cereus* is a transcriptional activator that controls expression of a number of known and potential virulence genes, including those encoding lecithinase, proteases and hemolysins. *B. anthracis* harbors homologues of many *plcR*-regulated genes, some of which are preceded by consensus sites for *PlcR*-binding. However, these genes do not appear to be highly expressed in

*B. anthracis* because *B. anthracis* lacks a functional PlcR protein. In an analogous manner, *B. cereus* and *B. thuringiensis* do not appear to harbor *atxA* or *acpA* homologues. However, these species contain homologues of the *atxA* target genes on the chromosome. Although the roles of the newly-identified *atxA*-regulated genes described here are not known, we view the species-specific regulators as important players in the transcriptomes of the species.

Our results represent a snapshot of the *B. anthracis* transcriptome for one time point in one specific growth condition. Certainly the transcription profile of *B. anthracis* will vary during growth in other conditions, including infection. It is noteworthy that in a mouse model for anthrax, a pXO1<sup>+</sup> pXO2<sup>-</sup> *atxA*-null mutant is avirulent and the antibody response to all three toxin proteins is decreased significantly. These data indicate that *atxA* function is important *in vivo*. No studies of *acpA* function during infection have been reported. In light of results presented here, studies of gene expression in *B. anthracis* and other plasmid-containing species should be interpreted with caution when cured strains are employed. It will be of interest to test the pXO1<sup>+</sup> pXO2<sup>+</sup> parent and regulatory gene mutants in animal models to determine relative virulence and to compare toxin and capsule synthesis *in vivo*. The significant cross-talk between the *B. anthracis* plasmids and chromosome is likely to be important for virulence and other aspects of *B. anthracis* physiology.

#### **SIGNIFICANCE:**

The identification of the *atxA* and *acpA* regulons provide researchers with a refined set of genes from the *B. anthracis* genome that represent novel virulence gene candidates that may be validated as potential therapeutic targets. It will be of interest to determine whether the proteins under *atxA* and *acpA* control are immunoreactive with sera from infected animals. The data generated also establish the first step in defining the *atxA/acpA* regulatory circuitry that likewise provides a unique basis and opportunity for therapeutic approaches in which attempts to "short circuit" the proper regulation of *atxA* and *acpA* target genes by interfering with their expression and functional impact to the cells virulence potential.

**PATENT INFORMATION:** none

**AWARD INFORMATION:** none

#### **PUBLICATIONS AND ABSTRACTS:**

1. Bourgogne, A., M. Drysdale, S.G. Hilsenbeck, **Peterson, S.N.**, T.M. Koehler (2003). Global Effects of Virulence Gene Regulators in a *Bacillus anthracis* Strain with both Virulence Plasmids. *Infection & Immunity*, **71**: 2736-2742.